

Claims

1. A method for producing a protein library, comprising the steps of:
- a) providing a population of RNA molecules, each of which comprises a translation initiation sequence and a start codon operably linked to a protein coding sequence and each of which is operably linked to a peptide acceptor at the 3' end of said protein coding sequence;
- 5 b) in vitro translating said protein coding sequences to produce a population of RNA-protein fusions; and
- c) further incubating said population of RNA-protein fusions under high salt conditions, thereby producing a protein library.
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2. A method for producing a DNA library, comprising the steps of:
- a) providing a population of RNA molecules, each of which comprises a translation initiation sequence and a start codon operably linked to a protein coding sequence and each of which is operably linked to a peptide acceptor at the 3' end of said protein coding sequence;
- 15 b) in vitro translating said protein coding sequences to produce a population of RNA-protein fusions;
- c) further incubating said population of RNA-protein fusions under high salt conditions; and
- 20 d) generating from each of said RNA portions of said fusions a DNA molecule, thereby producing a DNA library.
3. A method for the selection of a desired protein or nucleic acid encoding said protein, comprising the steps of:
- a) providing a population of candidate RNA molecules, each of which
- 25 comprises a translation initiation sequence and a start codon operably linked to a

candidate protein coding sequence and each of which is operably linked to a peptide acceptor at the 3' end of said candidate protein coding sequence;

b) in vitro translating said candidate protein coding sequences to produce a population of candidate RNA-protein fusions;

5 c) further incubating said population of candidate RNA-protein fusions under high salt conditions, thereby producing a protein library; and

d) selecting a desired RNA-protein fusion, thereby selecting said desired protein and said nucleic acid encoding said protein.

10 4. The method of any of claims 1-3, wherein said high salt comprises a monovalent cation.

5. The method of claim 4, wherein said monovalent cation is at a concentration of between approximately 125 mM - 1.5 M.

6. The method of claim 5, wherein said monovalent cation is at a concentration of between approximately 300 mM - 600 mM.

15 7. The method of claim 4, wherein said monovalent cation is K^+ or NH_4^+ .

8. The method of claim 4, wherein said monovalent cation is Na^+ .

9. The method of claim 7, wherein said incubating step is carried out at approximately room temperature.

20 10. The method of any of claims 1-3, wherein said high salt comprises a divalent cation.

11. The method of claim 10, wherein said divalent cation is at a concentration of between approximately 25 mM - 200 mM.

12. The method of claim 10, wherein said divalent cation is Mg^{+2} .

13. The method of any of claims 1-3, wherein said high salt comprises both a
5 monovalent and a divalent cation.

14. The method of any of claims 1-3, wherein each of said RNA molecules further comprises a pause sequence or further comprises a DNA or DNA analog sequence covalently bonded to the 3' end of said RNA molecule.

15. The method of claim 14, wherein said pause sequence or said DNA or
10 DNA analog sequence is of a length sufficient to span the distance between the decoding site and the peptidyl transfer center of a ribosome.

16. The method of claim 14, wherein said pause sequence or said DNA or DNA analog sequence is approximately 60-70 A° in length.

17. The method of claim 14, wherein said pause sequence or said DNA or
15 DNA analog sequence is less than approximately 80 nucleotides in length.

18. The method of claim 14, wherein said pause sequence or said DNA or DNA analog sequence is less than approximately 45 nucleotides in length.

19. The method of claim 14, wherein said pause sequence or said DNA or DNA analog sequence is between approximately 21-30 nucleotides in length.

20. The method of claim 14, wherein said pause sequence or said DNA or DNA analog sequence is joined to said RNA molecule using a DNA splint.

21. The method of claim 14, wherein said pause sequence or said DNA or DNA analog sequence comprises a non-nucleotide moiety.

5 22. The method of claim 14, wherein said non-nucleotide moiety is one or more $\text{HO}(\text{CH}_2\text{CH}_2\text{O})_3\text{PO}_2$ moieties.

23. The method of any of claims 1-3, wherein said RNA-protein fusion further comprises a nucleic acid or nucleic acid analog sequence positioned proximal to said peptide acceptor which increases flexibility.

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A4

add
B1